The Accumulation of Heavy Metals (Pb and Cd) of the Plant Family Zingiberaceae at Srinakarind Dam, Kanchanaburi Province

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Abstract

Indigenous plants and herbs have been consumed by local people in Thailand for past decades. The use of these plants beside taste and aroma for the dish, it is also used for its medicinal properties. In addition, the native plants are inexpensive and readily available, so most of the users are not aware of some toxin that could present such as phytotoxin and heavy metals. Heavy metals including lead (Pb) and Cadmium (Cd) can be occurred naturally or from external contamination. The accumulation of heavy metal can be found at any part of the plants such as leaf, root and stem. The aim of the study was to examine the level of heavy metal in *Zingiber cassumunar* Roxb., *Alpinia malaccensis* and *Zingiber citriodorum* which cultivated in the Royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, Kanchanaburi Province, Thailand where forest expose to minimum human activities leading less contaminated from fertilizers and pesticides. Heavy metals content were analyzed by Inductively coupled plasma (ICP). The results indicated that the content of Pb in *Z. casssumunar* Roxb., *A. laccensis* and *Z. citriodorum* were 0.074 ± 0.012 $mg.kg^{-1}$, 0.025 ± 0.001 $mg.kg^{-1}$ and 0.127 ± 0.01 $mg.kg^{-1}$ respectively. Considering Cd concentration, *Z. citriodorum* had the highest concentration (0.057 ± 0.00 $mg.kg^{-1}$) followed by *Z. casssumunar* Roxb (0.043±0.00 $mg.kg^{-1}$), and *A. laccensis* (0.041 ± 0.02 $mg.kg^{-1}$). However, the presence of those heavy metals were below Maximum Permitted Level (Pb ≤ 1 $mg.kg^{-1}$, Cd ≤ 0.3 $mg.kg^{-1}$) indicating that those roots plants were safe to consume. It can be seen that, even the root plants are belong to same family, they accumulates heavy metal differently. This could be due to the thickness of the plant's cell wall and the location of plant habitat. From the results, the content of both Pb and Cd in *Z. cassumunar* Roxb., *A. malaccensis* and *Z. citriodorum* were considered as in low level, though they grow in reserved area. It is indicated that there are accumulation of heavy metal which occur naturally in nature. Thus, they are safe for consumption.

Keywords: *Alpinia malaccensis*, Cadmium, Lead, Zingiberaceae family*, Zingiber cassumunar* Roxb *, Zingiber citriodorum*

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การสะสมของโลหะหนัก (ตะกั่ว และแคดเมียม) ของพืชตระกูล Zingiberaceae ในพื้นที่เขื่อนศรีนครินทร์ จังหวัดกาญจนบุรี

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บทคัดย่อ

ทศวรรษที่ผ่านมาพืชพื้นเมืองและสมุนไพรได้รับความนิยมในการนำมาบริโภคโดยคนในท้องถิ่นในประเทศไทย พืชเหล่านี้มีรสชาติและกลิ่นหอมเหมาะสำหรับนำมาปรุงอาหาร และใช้เป็นสมุนไพร นอกจากนี้พืชพื้นเมืองมีราคาไม่แพงและสามารถหาได้ง่ายในท้องถิ่น ทำให้มีการใช้พืชสมุนไพรเหล่านี้อย่างกว้างขวางโดยผู้ใช้ไม่ได้ตระหนักถึงพิษที่อาจจะได้รับจากพืช เช่น สารพิษและโลหะหนัก เช่นตะกั่ว (Pb) และแคดเมียม (Cd) เป็นต้น ซึ่งสามารถเกิดขึ้นตามธรรมชาติหรือจากการปนเปื้อนในสิ่งแวดล้อม พืชสามารถสะสมโลหะหนักไว้ในส่วนต่างๆของต้นได้ เช่นใบ ราก และลำต้น วัตถุประสงค์ของการศึกษานี้คือจะศึกษาปริมาณของโลหะหนักในไพล (*Zingiber cassumunar* Roxb.) ข่าป่า (*Alpinia malaccensis*) และกระชายพราน (*Zingiber citriodorum*) ซึ่งเป็นพืชที่ปลูกในโครงการพระราชดำริขอสมเด็จพระเทพรัตนราชสุดาฯ สยามบรมราชกุมารี จังหวัดกาญจนบุรี ซึ่งเป็นพื้นที่ป่าอนุรักษ์ที่ไม่ถูกรบกวนจากมลพิษหรือการกระทำของมนุษย์ เช่น การใช้ปุ๋ยและยาฆ่าแมลง โดยวิเคราะห์โลหะหนักด้วย Inductively coupled plasma (ICP) ผลการวิจัยพบว่าปริมาณของตะกั่วในไพล ข่าป่า และกระชายพรานเท่ากับ 0.074 ± 0.012 $mg.kg^{-1}$, 0.025 ± 0.001 $mg.kg^{-1}$ และ 0.127 ± 0.01 $mg.kg^{-1}$ ตามลำดับ ในขณะที่การสะสมของแคดเมี่ยมในกระชายพรานมีความเข้มข้นสูงสุด (0.057 ± 0.00 $mg.kg^{-1}$) ตามด้วยไพล (0.043 ± 0.00 $mg.kg^{-1}$) และ ข่าป่า (0.041 ± 0.02 $mg.kg^{-1}$) ซึ่งปริมาณของโลหะหนักที่พบนั้นต่ำกว่าระดับสูงสุดที่อนุญาต (Pb ≤ 1 $mg.kg^{-1}$, Cd ≤ 0.3 $mg.kg^{-1}$) แสดงให้เห็นว่าพืชเหล่านี้มีความปลอดภัยในการบริโภค จะเห็นได้ว่าพืชตระกูลเดียวกันสามารถสะสมโลหะหนักได้แตกต่างกัน ซึ่งอาจเป็นเพราะความหนาของผนังเซลล์ของพืช และพื้นที่เพาะปลูก จากผลการศึกษานี้ชี้ให้เห็นว่าทั้งตะกั่วและแคดเมียมในไพล ข่า และกระชายพราน มีปริมาณต่ำแม้ว่าจะเกิดในพื้นที่ธรรมชาติห่างไกลเมือง ซึ่งแสดงให้เห็นว่าเป็นการสะสมของโลหะหนักที่เกิดขึ้นตามธรรมชาติ แต่อย่างไรก็ตามยังมีความปลอดภัยในการบริโภค

คำสำคัญ: ข่าป่า แคดเมียม ตะกั่ว พืชตระกูลข่า ไพล กระชายพราน

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Introduction

Local people usually use indigenous plants as food and traditional medicines. Currently, the trend of herb and food remedies has been increasing. This is one of the factors that increase the utilization of wild plants. Fresh consumption is a popular choice of local plant usage. However, fresh consumption could cause health adverse effects from natural phytotoxins, pathogens contamination and heavy metal accumulation in the plants.

Recently studies have related the accumulation of heavy metals in several herbal remedies. Okem *et al*. (2014)[1] reported that in South African medicinal plant such as *Rapenea melanophloeos* and *Alepidea amatymbic* have been contaminated with many toxic elements including lead (Pb), arsenic (As) and cadmium (Cd).

Heavy metal such as Pb and Cd are naturally contaminated in environment or by human activities such as industrial and cultivation. Soil is the main source of heavy metal contamination followed by water. Plant absorbs foods and nutrients from soil by diffusion through the rhizosphere[2]. This shows that the roots are the first part that contact with heavy metal and could accumulated heavy metal elements. The toxicity of heavy metal especially lead could cause renal dysfunction, nervous system disease and cross placenta to fetus[3] and cancer[4]. The chronic toxicity of cadmium is pulmonary diseases[5]. In addition, the IARC has classified cadmium as Group 1 carcinogens[6].

Zingiber cassumunar Roxb. (Plai), *Alpinia malaccensis* (Khapa) and *Zingiber citriodorum* (Krachaipran) are local plants that can be found abundantly in Kanchanaburi Province, Thailand. These plants belong to the same family, Zingiberaceae, (roots herb). The application of plants included drinks, food or medicines. The studies have found that all three plants exhibit medicinal properties such as anti-inflammatory and anti-virus. Furthermore, *Z. cassumunar* Roxb. (Plai) is used for its antimicrobial activity[7]. *A. malaccensis* (Khapa) showed strong antioxidant activity in hepatotoxicity induced mice[8].

Due to the good taste, aroma and health promoting function in Zingiberaceae family, it is still not sufficient to promote the usage of this plant family. Plant accumulates various compounds beside bioactive compounds such as phytotoxin and heavy metal. Therefore, before encourage the consumption of these plants, it is important to study the possible presence of phytotoxin or heavy metal in the plants. This is to ensure that safe consumption of plants and food security. The aim of this study was to study the presence of heavy metals including Pb and Cd in *Z. cassumunar* Roxb. (Plai), *A. malaccensis* (Khapa) and

Z. citriodorum (Krachaipran) in reserved area using ICP-OES-method.

Materials and Methods

Plants and chemicals

Z. cassumunar Roxb. (Plai), *A. malaccensis* (Khapa) and *Z. citriodorum* (Krachaipran) (Table 1) were collected from the Royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, Kanchanaburi Province, Thailand in May 2014.

Chemicals and reagents

Concentrated nitric acid (MERCK, Germany), Perchloric (HPO_4) (MERCK, Germany), cadmium standard solution 1000 mg/L (BDH, Spectrosol, England) and lead standard solution 1,000 mg/L (Merck, Germany).

Samples preparation

Only the edible part of samples was analyzed. The brown outer part of Plai was removed and the yellow inner flesh was used for analysis which similarly to Khapa. While Krachaipran, the light brow root was cleaned and used as a whole for sample analysis (Table 1). The edible part of each sample was collected, washed twice with tab water and rinsed with DI water. After that samples were freeze dried and blended to the powder.

Samples digestion

Approximately 0.5 g of dried samples were weighed and mixed with 5 mL of 65% HNO_3 (concentrated) and 1 mL of Perchloric (HPO_4) [5:1] in a teflon jar. Sample digestion was conducted at 100 °C, 9 h in hot air oven and cooled under the fume hood. Solution was filtered into volumetric flask and volume was adjusted to 25 ml with DI water. All sample solutions were run through Inductively Coupled Plasma Optical Emission spectrometry (ICP-OES) (PerkinElmer^{TM}, optima 4200 DV, USA) with WinLab32^{TM} software. Operational conditions used for determination are shown in Table 2.

Statistic analysis

Statistical analysis were performed in triplicate and data analyzed are mean ± SD subjected to One-Way ANOVA by using SPSS 19 (Statistical Package for the Social Science), significant different at $p < 0.05$.

Table 1 The medicinal plants (Zingiberaceae family) used in this study

Plant (scientific name)	Local name	Part used
Zingiber casssumunar Roxb. 	Plai (Cassumunar ginger, Bengal root)	Rhizome
Alpinia malaccensis 	Khapa (Galanga)	Rhizome
Zingiber citriodorum 	Krachaipran	Rhizome

(Faculty of pharmacy , Mahidol University, 2015; Agrofolio, 2015; Institute of Nutrition, Mahidol University, 2011)

Table 2 Operational parameters for determination of Pb and Cd by ICP-OES

Parameter	ICP-OES
Radio-frequency power (W)	1,400
Plasma gas flow rate (L/min)	15.0
Auxiliary gas flow rate (L/min)	0.2
Nebulizer gas flow rate (L/min)	0.8
Observation view	Axial
Analyte	
Pb	220.353
Cd	228.802

Operational parameters used in this experiment were shown in Table 2. Plasma and auxiliary flow rate were kept constant at 15.0 and 0.2 L/min respectively. In addition, Pb and Cd were observed at 220.353 and 228.802.

Results and discussion

It is widely accepted that a great deal about the accumulation of heavy metals are from the human[9]. Therefore, the area that is reserved and away from human activity is desirable for this study. Hence, the Royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, is selected. It is also the conservative area with no contaminated from fertilizer and pesticide area. The samples are Zingiberaceae family that is rhizomes herb (Table 1).

Root herbal has been used for a long time and can contaminated with varieties of metal depending on plant species, collecting site and bio-accumulation[10]. The result of this study showed that the edible part contain heavy metal.

The highest level of Pb was detected in *Z. citriodorum* (0.127 ± 0.01 mg/kg) followed by *Z. cassumunar* Roxb. (0.074 ± 0.012mg/kg) and the lowest was *A. malaccensis* (0.025 ± 0.001 mg/kg) (Figure 1).

Pb that found in three plants are significantly different ($p < 0.05$). Cecchi *et al.*[11] reported that Pb normally accumulated in top soil (about 8 inch from the surface) and decreases for deeper soil layer. Therefore, root plants that stay on soil can contain Pb in this part.

Although all three plants in this study are in the same family, their abilities to accumulated Pb are different which depends on various factors such as soil pH[12], surface of root, immature of plant and the thickness of cell wall at root because metal diffused through cell wall and stored in vacuole[13].

This study showed that the accumulation of lead in all three plants are significantly different ($p < 0.05$). The highest accumulation is Krachaipran followed by the Plai and Khapa, respectively However, the accumulation of cadmium in all three plants is no different.

Regarding Cd content, *Z cassumunar* Roxb., *A. malaccensis* and *Z. citriodorum* were, 0.043 ± 0.00 $mg.kg^{-1}$, 0.041 ± 0.02 $mg.kg^{-1}$ and 0.057 ± 0.00 $mg.kg^{-1}$, respectively (Figure 1). This finding is agreed with the finding to Cd in the rhizome of ginger, galangal, black galingale which are belong to Zingiberaceae family, the average is found 0.3 mg/kg at Nakhon Pathom Province[14]. Rhizome is the primary part of plant that contact with heavy metal. It is positioned on the ground which mineral and heavy metal are presences. In addition, the transportation of mineral and element is conducted through the root. Hence, this part is likely to have heavy metal accumulation[15]. Ginger rhizome which belong to the same family of plant samples contain similarly in high Pb concentration[16].

Normally, the uptake of Cd in plant is flow through cell wall of root similar to Pb. After that, Cd are transported to different parts of the plant by xylem and move to grain by phloem[17,18]. Generally, Cd can be found in any parts of the plants. However, in this study, Cd was detected in rhizome in small quantity.

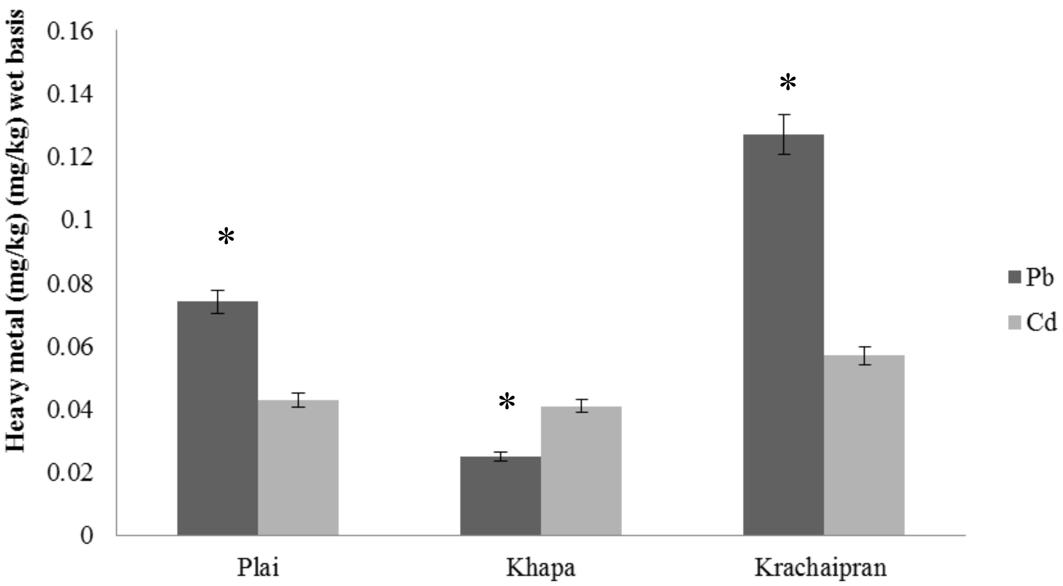

Figure 1 The content of Pb and Cd (mg/kg) wet basis in Plai (*Zingiber cassumunar* Roxb.), Khapa(*Alpinia malaccensis*) and Krachaipran (*Zingiber citriodorum*) edible part analysed by ICP-OES

Moreover, the accumulation of Cd are varied depends on species of plant or the expression of Zinc-regulated transporter Iron-regulated transporter protein (ZIP) and Heavy Metal ATPase 4 (HMA4). Those are the transporter regulation of Cd[19]. In addition, high levels of Pb and Cd can cause plant toxicity, growth retardation and affect the expression of the plants genotype[20]. The level of heavy metals in this study was considered to be safe to consume in moderate level (Thai FDA, 1986)[21]. According to FDA regulation, the concentration of Pb less than 1 $mg.kg^{-1}$[21] is safe. There is no specified limits of Cd in food but the standard heavy metal contamination in traditional medicine is limited to 0.3 mg/kg[22].The limits of Cd in food of FAO/WHO is defined in the PTWI (Provisional Tolerable Weekly Intake).

Conclusion

This study concluded that *Z. cassumunar* Roxb. (Plai), *A. malaccensis* (Khapa) and *Z. citriodorum* (Krachaipran) from Royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, Kanchanaburi Province, Thailand, contain safe level of heavy metal accumulation for consume. However, the higher intake of these plants as traditional medicine will cause acute toxicity because the accumulation of heavy metals in the body. It depends on the characteristics of the plants, the edible part can accumulate heavy metals in high level. In addition, soil is a major source of heavy metals contamination. Therefore, growing location and human factors should be carefully observed to acquire safe food.

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